Replacement Sheet

MKK4 (cDNA accession number: AB015315)

Wild-type cDNA sequence from start *to* end *codons
(1101 base pairs including the end TAG):*

atgagaccgattcaatcgcctccaggagtttccgttccggtgaaaagccgtccccgtcg
ccgtcctgatcttaccttaccgcttcctcaacgcgatgtttctctcgctgtacctcttc
ctctcccacctacttccggtggttccggtggctctagtggatctgcgccgtcttctggt
ggttcggcgtcttcaacgaacactaacagctccatagaagcgaagaactattcggattt
agtgagaggtaaccgtatcggaagcggagcaggtggaacggtatacaaagtgattcacc
gtccgagttctcgtctatatgcacttaaggtgatatacggtaaccacgaggagactgtg
agacgtcagatctgtagagagatcgagattttacgagatgtgaatcatccaaacgttgt
gaaatgtcacgagatgtttgatcagaacggtgagatccaggttttgcttgagtttatgg
ataaaggttctttagaaggtgctcatgtgtggaaagagcaacaattagctgatctatct
cgtcagattcttagtggtttagcttatctccatagccgtcacatagttcatcgtgatat
caaaccatcgaatcttttgataaactctgctaaaaacgttaagattgctgattttggag
ttagtaggatcttggctcagactatggatccgtgtaattcatctgttggaaccattgct
tatatgagtcctgagaggattaacactgatttgaatcagggaaagtatgatggttatgc
tggagatatttggagcttaggtgttagcattttggagttttacttggggaggtttcctt
tccctgtgagtagacaaggtgattgggctagtcttatgtgtgccatttgtatgtctcag
cctccagaagctccagcgactgcgtcgccggagtttcggcattttatctcgtgttgctt
gcagagagaaccggggaaaaggaggagtgctatgcagctattgcagcatcctttcatat
taagagcaagtccgagccagaacaggtctcctcagaatctacatcaactcttgcctcct
cctcgtcctctgtcctcgtcttcttctccaaccacatag (SEQ ID NO.:3)

Wild-type protein sequence (366 aminoacids):

MRPIQSPPGVSVPVKSRPRRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGGSSGSAPSSG
GSASSTNTNSSIEAKNYSDLVRGNRIGSGAGGTVYKVIHRPSSRLYALKVIYGNHEETV
RRQICREIEILRDVNHPNVVKCHEMFDQNGEIQVLLEFMDKGSLEGAHVWKEQQLADLS
RQILSGLAYLHSRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQTMDPCNSSVGTIA
YMSPERINTDLNQGKYDGYAGDIWSLGVSILEFYLGRFPFPVSRQGDWASLMCAICMSQ
PPEAPATASPEFRHFISCCLQREPGKRRSAMQLLQHPFILRASPSQNRSPQNLHQLLPP
PRPLSSSSSPTT   (SEQ ID NO.:4)

FIGURE 4

Mutations rendering MKK4 constitutively active:

- modify Threonine (T) 224 to Aspartic Acid (D) by changing codon ACT into GAT
- modify Serine (S) 230 to Glutamic Acid (E) by changing codon TCA into GAA

The mutations were done by PCR using the primers (mutated base pairs in lower case, both are from 5' end to 3' end):

CTTGGCTCAGgaTATGGATCCGTGTAATgaATCTGTTGGAAC
TCCAACAGATtcATTACACGGATCCATAtcCTGAGCCAAG   (SEQ ID NO.3)

So the sequences after mutations are:

MKK4act mutant sequence from start *to* end *codons*
(1101 base pairs including the end TAG):

atgagaccgattcaatcgcctccaggagtttccgttccggtgaaaagccgtccccgtcgccgtcctgatct
taccttaccgcttcctcaacgcgatgtttctctcgctgtacctcttcctctcccacctacttccggtggtt
ccggtggctctagtggatctgcgccgtcttctggtggttcggcgtcttcaacgaacactaacagctccata
gaagcgaagaactattcggatttagtgagaggtaaccgtatcggaagcggagcaggtggaacggtatacaa
agtgattcaccgtccgagttctcgtctatatgcacttaaggtgatatacggtaaccacgaggagactgtga
gacgtcagatctgtagagagatcgagattttacgagatgtgaatcatccaaacgttgtgaaatgtcacgag
atgtttgatcagaacggtgagatccaggtttttgcttgagtttatggataaaggttctttagaaggtgctca
tgtgtggaaagagcaacaattagctgatctatctcgtcagattcttagtggtttagcttatctccatagcc
gtcacatagttcatcgtgatatcaaaccatcgaatcttttgataaactctgctaaaaacgttaagattgct
gattttggagttagtaggatcttggctcaggatatggatccgtgtaatgaatctgttggaaccattgctta
tatgagtcctgagaggattaacactgatttgaatcagggaaagtatgatggttatgctggagatatttgga
gcttaggtgttagcattttggagtttttacttggggaggtttcctttccctgtgagtagacaaggtgattgg
gctagtcttatgtgtgccatttgtatgtctcagcctccagaagctccagcgactgcgtcgccggagtttcg
gcattttatctcgtgttgcttgcagagagaaccggggaaaaggaggagtgctatgcagctattgcagcatc
ctttcatattaagagcaagtccgagccagaacaggtctcctcagaatctacatcaactcttgcctcctcct
cgtcctctgtcctcgtcttcttctccaaccacatag (SEQ ID NO.:6)

MKK4act mutant protein sequence (366 aminoacids):

MRPIQSPPGVSVPVKSRPRRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGGSSGSAPSSGGSASSTNTNSSI
EAKNYSDLVRGNRIGSGAGGTVYKVIHRPSSRLYALKVIYGNHEETVRRQICREIEILRDVNHPNVVKCHE
MFDQNGEIQVLLEFMDKGSLEGAHVWKEQQLADLSRQILSGLAYLHSRHIVHRDIKPSNLLINSAKNVKIA
DFGVSRILAQDMDPCNESVGTIAYMSPERINTDLNQGKYDGYAGDIWSLGVSILEFYLGRFPFPVSRQGDW
ASLMCAICMSQPPEAPATASPEFRHFISCCLQREPGKRRSAMQLLQHPFILRASPSQNRSPQNLHQLLPPP
RPLSSSSSPTT (SEQ ID NO.:7)

FIGURE 5

MKK5 (cDNA accession number: AB015316)

Wild-type cDNA sequence from start *to* end *codons*
(1047 base pairs including the end TAG):

atgaaaccgattcaatctccttctggagtagcttcacctatgaagaaccgtttacgcaa
acgtcctgacctaagcttaccactcccacaccgcgacgtcgctctcgccgtacctctcc
ctctcccacctccttcttcctcttcatccgctccggcgtcttcctccgcgatctcaacc
aacatctccgccgctaaaagcttatccgagctagaacgagtgaaccgaatcggaagcgg
agccggaggaacggtttacaaagtaatccacactccgacgtcacgtcctttcgctctca
aagtgatttacggaaaccacgaagataccgtgagacgtcagatctgtagagagatcgag
atcttaagaagtgttgatcatccaaacgttgtgaaatgtcacgatatgtttgatcataa
cggtgagatccaggttttgcttgagtttatggatcaaggatctcttgaaggagctcata
tatggcaagaacaggaattagctgatctctctcgtcagattcttagtggattagcttat
cttcatcgtcgtcatatcgttcatcgtgatatcaaaccttcgaatctccttataaactc
agctaaaaatgtgaaaattgctgattttggtgtgagtaggatcttggcacaaacaatgg
atccttgtaattcatctgttggtactattgcttatatgagtcctgagaggattaatact
gatttgaatcatggtcgttacgatggttatgctggagatgtttggagtttaggtgttag
tatcttggagtttttacttggggaggtttccttttgctgtgagtagacaaggtgattggg
ctagtcttatgtgtgctatttgtatgtctcagccacctgaagctccggctacggcgtct
caggagtttcgtcactttgtttcttgttgtttacagagtgatcctcctaagagatggtc
agctcaacagctttgcagcatcctttcatacttaaagctaccggtggtcctaatctcc
gtcaaatgttgccgccgcctcgtcctcttccttctgcctcttag (SEQ ID NO.:8)

Wild-type protein sequence (348 aminoacids):

MKPIQSPSGVASPMKNRLRKRPDLSLPLPHRDVALAVPLPLPPPSSSSSAPASSSAIST
NISAAKSLSELERVNRIGSGAGGTVYKVIHTPTSRPFALKVIYGNHEDTVRRQICREIE
ILRSVDHPNVVKCHDMFDHNGEIQVLLEFMDQGSLEGAHIWQEQELADLSRQILSGLAY
LHRRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQTMDPCNSSVGTIAYMSPERINT
DLNHGRYDGYAGDVWSLGVSILEFYLGRFPFAVSRQGDWASLMCAICMSQPPEAPATAS
QEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATGGPNLRQMLPPRPLPSAS (SEQ
ID NO.:9)

FIGURE 6

Mutations rendering MKK5 constitutively active:

- modify Threonine (T) 215 to Glutamic Acid (E) by changing codon ACA into GAA
- modify Serine (S) 221 to Glutamic Acid (E) by changing codon TCA into GAA

The mutations were done by PCR using the primers (mutated base pairs in lower case, both are from 5' end to 3' end):

CTTGGCACAAgaAATGGATCCTTGTAATgaATCTGTTGGT
ACCAACAGATtcATTACAAGGATCCATTtcTTGTGCCAAG   (SEQ ID NO.:10)

So the sequences after mutations are:

*MKK5act mutant cDNA sequence from start to end codons
(1047 base pairs including the end TAG):*

atgaaaccgattcaatctccttctggagtagcttcacctatgaagaaccgtttacgcaa
acgtcctgacctaagcttaccactcccacaccgcgacgtcgctctcgccgtacctctcc
ctctcccacctccttcttcctcttcatccgctccggcgtcttcctccgcgatctcaacc
aacatctccgccgctaaaagcttatccgagctagaacgagtgaaccgaatcggaagcgg
agccggaggaacggtttacaaagtaatccacactccgacgtcacgtcctttcgctctca
aagtgatttacggaaaccacgaagataccgtgagacgtcagatctgtagagagatcgag
atcttaagaagtgttgatcatccaaacgttgtgaaatgtcacgatatgtttgatcataa
cggtgagatccaggttttgcttgagtttatggatcaaggatctcttgaaggagctcata
tatggcaagaacaggaattagctgatctctctcgtcagattcttagtggattagcttat
cttcatcgtcgtcatatcgttcatcgtgatatcaaaccttcgaatctccttataaactc
agctaaaaatgtgaaaattgctgattttggtgtgagtaggatcttggcacaagaaatgg
atccttgtaatgaatctgttggtactattgcttatatgagtcctgagaggattaatact
gatttgaatcatggtcgttacgatggttatgctggagatgtttggagtttaggtgttag
tatcttggagttttacttggggaggtttccttttgctgtgagtagacaaggtgattggg
ctagtcttatgtgtgctatttgtatgtctcagccacctgaagctccggctacggcgtct
caggagtttcgtcactttgtttcttgttgtttacagagtgatcctcctaagagatggtc
agctcaacagcttttgcagcatcctttcatacttaaagctaccggtggtcctaatctcc
gtcaaatgttgccgccgcctcgtcctcttccttctgcctcttag (SEQ ID
NO.:11)

MKK5act mutant protein sequence (348 aminoacids):

MKPIQSPSGVASPMKNRLRKRPDLSLPLPHRDVALAVPLPLPPPSSSSSAPASSSAIST
NISAAKSLSELERVNRIGSGAGGTVYKVIHTPTSRPFALKVIYGNHEDTVRRQICREIE
ILRSVDHPNVVKCHDMFDHNGEIQVLLEFMDQGSLEGAHIWQEQELADLSRQILSGLAY
LHRRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQEMDPCNESVGTIAYMSPERINT
DLNHGRYDGYAGDVWSLGVSILEFYLGRFPFAVSRQGDWASLMCAICMSQPPEAPATAS
QEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATGGPNLRQMLPPPRPLPSAS   (SEQ
ID NO.:12)

FIGURE 7

Alignment of MKK4 and MKK5 wild-type:

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MKK4  MRPIQSPPGVSVPVKSRPRRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGG
MKK5  MKPIQSPSGVASPMKNRLRKRPDLSLPLPHRDVALAVPLPLPPPS-----

MKK4  SSGSAPSSGGSASSTNTNSSIEAKNYSDLVRGNRIGSGAGGTVYKVIHRP
MKK5  SSSSAPASS-SAISTNIS---AAKSLSELERVNRIGSGAGGTVYKVIHTP

MKK4  SSRLYALKVIYGNHEETVRRQICREIEILRDVNHPNVVKCHEMFDQNGEI
MKK5  TSRPFALKVIYGNHEDTVRRQICREIEILRSVDHPNVVKCHDMFDHNGEI

MKK4  QVLLEFMDKGSLEGAHVWKEQQLADLSRQILSGLAYLHSRHIVHRDIKPS
MKK5  QVLLEFMDQGSLEGAHIWQEQELADLSRQILSGLAYLHRRHIVHRDIKPS

MKK4  NLLINSAKNVKIADFGVSRILAQTMDPCNSSVGTIAYMSPERINTDLNQG
MKK5  NLLINSAKNVKIADFGVSRILAQTMDPCNSSVGTIAYMSPERINTDLNHG

MKK4  KYDGYAGDIWSLGVSILEFYLGRFPFPVSRQGDWASLMCAICMSQPPEAP
MKK5  RYDGYAGDVWSLGVSILEFYLGRFPFAVSRQGDWASLMCAICMSQPPEAP

MKK4  ATASPEFRHFISCCLQREPGKRRSAMQLLQHPFILRASPSQNRSPQNLHQ
MKK5  ATASQEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATG----GP-NLRQ

MKK4  LLPPPRPLSSSSSPTT      (SEQ ID NO.:13)
MKK5  MLPPPRPLPSAS----      (SEQ ID NO.:14)
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FIGURE 8